

WHAT IS CLAIMED IS:

1. A method for making a nucleic acid molecule comprising
  - (a) mixing a nucleic acid template with (i) one or more polypeptides having polymerase activity and/or reverse transcriptase activity and (ii) a primer-adapter nucleic acid molecule; and
  - (b) incubating said mixture under conditions sufficient to make a first nucleic acid molecule complementary to all or a portion of said template, wherein said primer-adapter nucleic acid molecule comprises one or more ligands and one or more cleavage sites.
2. The method of claim 1, wherein said first nucleic acid molecule comprises said primer-adapter nucleic acid molecule.
3. The method of claim 1, wherein said template is RNA or DNA.
4. The method of claim 3, wherein said RNA is a mRNA or a polyA+ RNA molecule.
5. The method of claim 1, wherein said first nucleic acid molecule is RNA or DNA.
6. The method of claim 1, wherein said polypeptide is selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase, an Avian Myeloblastosis Virus (AMV) reverse transcriptase, a *Tne* DNA polymerase, a *Tma* DNA polymerase, a *Taq* DNA polymerase, a *Tth* DNA polymerase, a *Tli* or VENT™ DNA polymerase, a *Pfu* or DEEPVENT™ DNA polymerase, a *Pwo* DNA polymerase, a *Bst* DNA polymerase, a *Sac* DNA polymerase, a *Tac* DNA

(xxix) a transferrin receptor; (xxx)  $\text{Fe}^{+++}$ ; (xxxi) polymyxin B or endotoxin-neutralizing protein (ENP); (xxxii) an enzyme-specific substrate; (xxxiii) protein A, protein G, a cell-surface Fc receptor or an antibody-specific antigen; and (xxxiv) avidin and streptavidin.

5            11.    The method of claim 1, wherein said cleavage site is a restriction endonuclease cleavage site or an endonuclease cleavage site.

10           ~~12.~~    The method of claim 2, ~~said method further comprising incubating~~ said first nucleic acid molecule under conditions sufficient to make a second nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule.

             13.    The method of claim 12, wherein said second nucleic acid molecule is a RNA or a DNA molecule.

             14.    The method of claim 12, wherein said first and said second nucleic acid molecules form a double-stranded nucleic acid molecule.

15           15.    The method of claim 14, wherein said double-stranded nucleic acid molecule is a double-stranded cDNA molecule.

~~16.~~    The method of claim ~~12~~, wherein said incubation step comprises mixing said first nucleic acid molecule with a DNA polymerase, one or more nucleotides and one or more primers.

20           ~~17.~~    The method of claim ~~16~~, wherein said primers are primer-adapters which comprise one or more ligands and one or more cleavage sites.

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18. The method of claim 2, said method further comprising binding one or more of said ligands to one or more haptens thereby forming a nucleic acid-ligand-hapten complex.

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19. The method of claim 12, said method further comprising binding one or more of said ligands to one or more haptens thereby forming a nucleic acid-ligand-hapten complex.

20. The method of claim 18 or claim 19, said method further comprising isolating said nucleic acid molecule from said complex by cleavage of one or more of said cleavage sites.

21. The method of claim 20, wherein said nucleic acid molecule is a double-stranded or a single-stranded nucleic acid molecule.

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22. The method of claim 18 or claim 19, wherein said one or more haptens are bound to a solid support.

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23. The method of claim 22, wherein said solid support is selected from the group consisting of nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, a latex bead, a magnetic bead, a paramagnetic bead, a superparamagnetic bead and a microtitre plate.

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24. The method of claim 18 or claim 19, wherein said one or more haptens are selected from the group consisting of (i) avidin and streptavidin; (ii) protein A, protein G, a cell-surface Fc receptor or an antibody-specific antigen; (iii) an enzyme-specific substrate; (iv) polymyxin B or endotoxin-neutralizing protein (ENP); (v)  $\text{Fe}^{+++}$ ; (vi) a transferrin receptor; (vii) an insulin

receptor; (viii) a cytokine (*e.g.*, growth factor, interleukin or colony-stimulating factor) receptor; (ix) CD4; (x) spectrin or fodrin; (xi) ICAM-1 or ICAM-2; (xii) C3bi, fibrinogen or Factor X; (xiii) ankyrin; (xiv) integrins  $\alpha_1\beta_1$ ,  $\alpha_2\beta_1$ ,  $\alpha_3\beta_1$ ,  $\alpha_6\beta_1$ ,  $\alpha_7\beta_1$  and  $\alpha_6\beta_5$ ; (xv) integrins  $\alpha_1\beta_1$ ,  $\alpha_2\beta_1$ ,  $\alpha_3\beta_1$  and  $\alpha_v\beta_3$ ; (xvi) integrins  $\alpha_3\beta_1$ ,  $\alpha_4\beta_1$ ,  $\alpha_4\beta_7$ ,  $\alpha_5\beta_1$ ,  $\alpha_v\beta_1$ ,  $\alpha_{IIB}\beta_3$ ,  $\alpha_v\beta_3$  and  $\alpha_v\beta_6$ ; (xvii) integrins  $\alpha_v\beta_1$  and  $\alpha_v\beta_3$ ; (xviii) vitronectin; ~~(xix) fibronectin~~; (xx) collagen; (xxi) laminin; (xxii) glycophorin; (xxiii) Mac-1; (xxiv) LFA-1; (xxv)  $\beta$ -actin; (xxvi) gp120; (xxvii) cytokines (growth factors, interleukins or colony-stimulating factors); (xxviii) insulin; (xxix) ferrotransferrin; (xxx) apotransferrin; (xxxi) lipopolysaccharide; (xxxii) an enzyme; (xxxiii) an antibody; and (xxxiv) biotin.

25. The method of ~~claim 2~~, said method further comprising amplifying said first nucleic acid molecule.

26. The method of claim 25, wherein said amplification is accomplished by a method comprising incubating said first nucleic acid molecule with a DNA polymerase, one or more nucleotides and one or more primers.

27. The method of claim 26, wherein said primers are primer-adapters.

28. The method of ~~claim 12~~, said method further comprising amplifying said first and second nucleic acid molecules.

29. The method of claim 28, wherein said amplification is accomplished by a method comprising

(a) contacting said first nucleic acid molecule with a first primer-adapter which is complementary to a portion of said first nucleic acid molecule, and a second nucleic acid molecule with a second primer-adapter which is

34. The nucleic acid molecule of claim 33, wherein said one or more cleavage sites allows removal of said one or more ligands from said nucleic acid molecule.

polymerase, a *Tfi/Tub* DNA polymerase, a *Tru* DNA polymerase, a DYNAZYME™ DNA polymerase, an *Mth* DNA polymerase, a Rous Associated Virus (RAV) reverse transcriptase, a Myeloblastosis Associated Virus (MAV) reverse transcriptase, a Human Immunodeficiency Virus (HIV) reverse transcriptase, a retroviral reverse transcriptase, a retrotransposon reverse transcriptase, a hepatitis B virus reverse transcriptase, a cauliflower mosaic virus reverse transcriptase, a bacterial reverse transcriptase and mutants, variants and derivatives thereof.

7. The method of claim 4, wherein said first nucleic acid molecule is a cDNA molecule.

8. The method of claim 1, wherein said cleavage site allows removal of at least one of said ligands from said primer-adaptor nucleic acid molecule.

9. The method of claim 2, wherein said cleavage site allows removal of at least one of said ligands from said first nucleic acid molecule.

10. The method of claim 1, wherein said ligand molecule is selected from the group consisting of (i) biotin; (ii) an antibody; (iii) an enzyme; (iv) lipopolysaccharide; (v) apotransferrin; (vi) ferrotransferrin; (vii) insulin; (viii) cytokines (growth factors, interleukins or colony-stimulating factors); (ix) gp120; (x)  $\beta$ -actin; (xi) LFA-1; (xii) Mac-1; (xiii) glycophorin; (xiv) laminin; (xv) collagen; (xvi) fibronectin; (xvii) vitronectin; (xviii) integrins  $\alpha_v\beta_1$  and  $\alpha_v\beta_3$ ; (xix) integrins  $\alpha_3\beta_1$ ,  $\alpha_4\beta_1$ ,  $\alpha_4\beta_7$ ,  $\alpha_5\beta_1$ ,  $\alpha_v\beta_1$ ,  $\alpha_{IIB}\beta_3$ ,  $\alpha_v\beta_3$  and  $\alpha_v\beta_6$ ; (xx) integrins  $\alpha_1\beta_1$ ,  $\alpha_2\beta_1$ ,  $\alpha_3\beta_1$  and  $\alpha_v\beta_3$ ; (xxi) integrins  $\alpha_1\beta_1$ ,  $\alpha_2\beta_1$ ,  $\alpha_3\beta_1$ ,  $\alpha_6\beta_1$ ,  $\alpha_7\beta_1$  and  $\alpha_6\beta_5$ ; (xxii) ankyrin; (xxiii) C3bi, fibrinogen or Factor X; (xxiv) ICAM-1 or ICAM-2; (xxv) spectrin or fodrin; (xxvi) CD4; (xxvii) a cytokine (e.g., growth factor, interleukin or colony-stimulating factor) receptor; (xxviii) an insulin receptor;

~~molecule of claim 33, wherein  
 molecule of claim 35, wherein  
 port.~~

molecule of claim 35, wherein

37. The nucleic acid restriction endonuclease

38. The nucleic acid is double-stranded

39. The nucleic acid is a DNA molecule,

40. A nucleic acid

2.

acid mol  
or singl  
acid mol

acid mol  
a RNA

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(a) mixing an mRNA template with a polypeptide having reverse transcriptase activity and a primer-adaptor nucleic acid molecule, said primer-adaptor molecule comprising one or more ligands and one or more cleavage sites;

(c) binding said DNA-primer-adaptor molecule to a solid support through a ligand-hapten interaction; and

45. The method of claim 44, said method further comprising making a second DNA molecule complementary to all or a portion of said first DNA molecule.

47. The method of claim 46, wherein said second DNA molecule comprises a primer-adaptor molecule.

49. A method for producing a cDNA molecule, said method comprising



(b) incubating said first DNA molecule with a primer-adapter molecule, wherein said primer-adapter molecule comprises one or more ligands and one or more cleavage sites, under conditions sufficient to form a double-stranded DNA molecule comprising a primer-adapter molecule;

(d) isolating said double-stranded DNA molecule from said solid support by cleaving said one or more cleavage sites.

50. The method of claim 49, wherein said method is used to prepare a cDNA library from a mRNA sample.

51. A method for isolating a mRNA molecule, said method comprising

(a) mixing a RNA sample with a primer-adapter molecule which hybridizes to mRNA, wherein said primer-adapter molecule comprises one or more ligands and one or more cleavage sites, thereby forming a mRNA-primer-adapter molecule;

(b) binding said mRNA-primer-adaptor molecule to a solid support through a ligand-hapten interaction; and

(c) isolating said mRNA molecule from said solid support by cleaving said one or more cleavage sites;

52. The method of claim 51, wherein said primer-adaptor molecule comprises oligo(dT).

53. A method for isolating one or more desired nucleic acid molecules from a population of nucleic acid molecules comprising

(a) mixing said population of nucleic acid molecules with one or more target-specific primer-adapter molecules;

(b) incubating said mixture under conditions sufficient to bind said primer-adapter molecules to said desired nucleic acid molecules; and

(c) isolating one or more of said desired nucleic acid molecules.

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